

Reversing the deleterious effects of aging on neuronal communication and behavior: beneficial properties of fruit polyphenolic compounds¹⁻⁴

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ABSTRACT

Despite elegant research involving molecular biology studies and determination of the genetic mechanisms of aging, practical information on how to forestall or reverse the deleterious effects of aging may be years away. If this is the case, then it is prudent to try to establish other methods that can be used now to alter the course of aging. Numerous epidemiologic studies have indicated that individuals who consume diets containing large amounts of fruits and vegetables may reduce their risk for developing age-related diseases such as Alzheimer disease. Research from our laboratory suggested that dietary supplementation with fruit or vegetable extracts high in antioxidants (eg, blueberry or spinach extracts) might decrease the enhanced vulnerability to oxidative stress that occurs in aging. These reductions might be expressed as improvements in motor and cognitive behavior. Additional research suggested that mechanisms in addition to antioxidant and antiinflammatory activities might be involved in the beneficial effects of these extracts; the most important of these might be their ability to increase cellular signaling and neuronal communication. *Am J Clin Nutr* 2005;81(suppl):313S–6S.

KEY WORDS Aging, oxidative stress, inflammation, polyphenolic compounds, brain, signaling

INTRODUCTION

It is well known that many age-related behavioral changes in motor and cognitive performance occur even in the absence of specific, age-related, neurodegenerative diseases such as Alzheimer disease or Parkinson disease. Research discussed in this review suggests that the aged brain may provide a sensitive environment for the development of these diseases, leading to even more severe deficits in memory and/or motor function. This could result in increases in the number of elderly patients in need of hospitalization and/or custodial care. Therefore, unless some means is found to reduce these age-related decrements in neuronal function, health care costs will continue to increase exponentially. In both financial and human terms, it is extremely important to explore methods to retard or reverse age-related neuronal deficits and their subsequent behavioral manifestations. In this review, we describe the motor and cognitive deficits in behavior, show how these deficits are related to increased vulnerability to oxidative stress and inflammation, and describe

the possible role of nutritional supplementation with fruits containing large amounts of polyphenols, such as anthocyanins, in reversing or forestalling these deficits.

BEHAVIORAL DECREMENTS

A great deal of research indicates the occurrence of numerous neuronal and behavioral deficits during normal aging. These changes may include decrements in calcium homeostasis (1) and in the sensitivity of several receptor systems, most notably the dopaminergic (2, 3), muscarinic (4, 5), opioid (6), and adrenergic (7) receptor systems. These losses in neuronal function may be expressed ultimately as alterations in both cognitive (8) and motor (9) behaviors. The memory deficits are noted in cognitive tasks that require the use of spatial learning and memory (10), whereas motor function deficits may include decreases in balance, muscle strength, and coordination (9). These changes have been demonstrated in many studies and appear to occur among both animals (10) and human subjects (11). Alterations in memory appear to occur primarily in secondary memory systems and are reflected in the storage of newly acquired information (12). Deficits in motor performance are thought to be the result of alterations in the striatal dopamine or cerebellar systems, which show marked neurodegenerative changes with age (12, 13).

Research shows that the hippocampus mediates place learning, whereas the prefrontal cortex is critical for acquiring the rules that govern performance of particular tasks (ie, procedural knowledge). It appears that the dorsomedial striatum regulates spatial orientation that involves response and cue learning (14). Importantly for this review, substantial research indicates that factors such as oxidative stress (15) and inflammation (16, 17) may be major contributors to the behavioral decrements seen in aging.

In animal models, cognitive function is usually measured in a maze and motor function is measured with a battery of different

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tests, such as those that assess the time a rodent can remain on an accelerod (a slowly rotating rod). Maze procedures are used to assess learning (acquisition), working (short-term), and reference (long-term) memory functions. Reference memory is consistent among trials and is required for learning the general rules of any task (eg, swim to a platform) (18). In contrast, working memory describes the ability of the subject to hold specific information (places previously visited) in memory (18). Old rats were shown to have decrements in both reference and working memory in the Morris water maze (for review, see reference 15), the radial arm maze (for review, see reference 19), and the radial arm water maze (20).

OXIDATIVE STRESS AND INFLAMMATION IN AGING

Oxidative Stress

An abundance of data suggest that one of the most important factors mediating the deleterious effects of aging on behavior and neuronal function is oxidative stress (for review, see reference 21). The central nervous system appears to be especially vulnerable to the effects of oxidative stress, partially as a result of additional factors such as increases in the ratio of oxidized glutathione to total glutathione (22), significant lipofuscin accumulation (23) with bcl-2 increases (24), increases in membrane lipid peroxidation (25), reductions in glutamine synthetase (26), reductions in redox-active iron (23, 27), and alterations in membrane lipids (28). Importantly, in addition to these considerations, it has been shown that, not only is the central nervous system particularly vulnerable to oxidative stress, but this vulnerability increases during aging (for review, see references 29 and 30). Research has also shown that, in addition to the factors discussed above (eg, reductions in glutathione concentrations) (22), oxidative stress vulnerability in aging may be the result of 3 other factors, namely, alterations in the membrane microenvironment, alterations in calcium-buffering ability, and differential vulnerability of neurotransmitter receptors. Findings suggest that age-related changes in the neuronal plasma membrane molecular structure and physical properties (eg, increased rigidity) may play a role in increasing vulnerability to oxidative stress and inflammation (29, 31).

Inflammation

Evidence also suggests that inflammatory events in the central nervous system may play an important role in aging. By middle age, there is increased glial fibrillary acidic protein expression (32); in old age, expression occurs even in the absence of an inflammatory stimulus (33). In conjunction with this observation, it was reported that tumor necrosis factor- α is produced in greater amounts during cytotoxic reactions among elderly subjects (34) and that neuronal inhibition of glial activities may be lost during aging (35). Other studies reported increases in tumor necrosis factor- α and interleukin-6 concentrations in the sera of aged mice (36) and human subjects (37). In fact, it has been suggested that up-regulation of C-reactive protein may represent one factor in biological aging (38).

Another important point is that there may be important interactions of reactive oxygen species-generating agents and cytokines. For example, Manev and Uz (39) demonstrated increased sensitivity of senescent rats to central injections of kainic acid, an

excitotoxin that induces inflammatory reactions involving factors such as cytokines, complement proteins, and adhesion molecules. These may represent extracellular signals that act in concert with reactive oxygen species to initiate neuronal functional deficits and glial cell-neuron interactions (40-42). Therefore, it appears that the increases in sensitivity with respect to oxidative stress and inflammation that are observed in senescence may be involved in mediating age-related deficits. Similarly, it appears that treatments (eg, heavy-particle irradiation) that increase oxidative and/or inflammatory stressors may produce behavioral deficits that parallel those seen in aging (43-45). In addition, research has shown that the induction of neuronal/glial inflammation through central administration of lipopolysaccharide, a bacterial toxin that is a potent inflammatory agent, can reproduce many of the behavioral, inflammatory, neurochemical, and neuropathologic changes seen in the brains of patients with Alzheimer disease in some similar regions (eg, cingulate cortex), as well as producing changes in spatial learning and memory behavior (16, 17, 46, 47).

Previous studies (16, 17, 46-48) showed that chronic (28-37-d) infusion of lipopolysaccharide into the ventricles of young rats increased several markers of inflammation. These changes included but were not limited to increased numbers of activated astrocytes, increased numbers and densities of activated microglia (particularly within the hippocampus, cingulate cortex, and basal forebrain), increased concentrations of cytokines, degeneration of hippocampal pyramidal neurons, and impairment of working memory (16, 17, 46, 48).

EFFECTS OF FRUIT AND VEGETABLE SUPPLEMENTATION ON BEHAVIORAL AND NEURONAL DEFICITS IN AGING

Antioxidants have been studied for their effectiveness in reducing the deleterious effects of brain aging and behavior in many studies (49-51). Although many of those experiments yielded mixed results, research from our laboratory suggested that the combinations of antioxidant/antiinflammatory polyphenolic compounds found in fruits and vegetables may show efficacy in aging. Plants, including food plants (fruits and vegetables), synthesize a vast array of secondary chemical compounds that, although not involved in primary metabolism, are important for a variety of ecologic functions that enhance the plant's ability to survive. Interestingly, these compounds may be responsible for the multitude of beneficial effects that have been reported for fruits and vegetables, with an array of health-related bioactivities. Many studies (52-56) have suggested that the most important benefits of such compounds may be derived from their antioxidant and antiinflammatory properties. Until very recently, however, most dietary agents used to alter behavioral and neuronal effects with aging included nutritional supplements such as vitamins C and E, garlic (49), herbal supplements (eg, ginseng, *Ginkgo biloba*, and *ding lang*) (50), and dietary fatty acids (for review, see reference 51).

We thought that, given the considerable antioxidant/antiinflammatory potential of fruits and vegetables, they might show some efficacy in reducing the deleterious effects of aging on neuronal function and behavior. In our first study, we used fruits and vegetables identified as being high in antioxidant activity in the oxygen radical absorbance capacity assay (52-54) and showed that long-term (from 6 to 15 mo of age) feeding of F344



rats with an AIN-93 diet supplemented with strawberry or spinach extract (1–2% of the diet) or vitamin E (500 IU) retarded age-related decrements in cognitive and neuronal function compared to an AIN-93 diet alone. Results indicated that the supplemented diets could prevent the onset of age-related deficits in several indices (eg, cognitive behavior and Morris water maze performance) (55).

In a subsequent experiment (56), we found that dietary supplementation (for 8 wk) with spinach, strawberry, or blueberry extracts in an AIN-93 diet was effective in reversing cognitive deficits in Morris water maze performance function among aged (19-mo-old) F344 rats. However, only the blueberry supplement-treated group exhibited improved performance on tests of motor function. Specifically, the blueberry supplement-treated group displayed improved performance on rod walking and latency to falling from an accelerating rotarod. Both of these tests rely on balance and coordination. None of the other supplement-treated groups differed from the control group in these tasks.

Although examinations of reactive oxygen species production in brain tissue obtained from animals in the various diet groups indicated that the striata obtained from all of the supplement-treated groups exhibited significantly lower reactive oxygen species concentrations (assayed as 2',7'-dichlorofluorescein diacetate) than did the control group, these decreases did not appear to be sufficient to account for the observed significant beneficial effects of blueberry supplementation on motor and cognitive function. It was clear from this study (56) and a subsequent study (57) that the significant effects of blueberries on both motor and cognitive behavior might involve actions other than antioxidant or antiinflammatory activities. Research from several sources suggests that at least some of these actions may include alterations in signaling. It is known that flavonoids can have potent effects on cell signaling. For example, delphinidin inhibits endothelial cell proliferation and cell cycle progression through extracellular signal-regulated kinase (ERK)-1/2 activation (58), whereas grape seed proanthocyanidin can reduce ischemia/reperfusion-induced activation of JNK-1 and c-Jun and reduce cardiomyocyte apoptosis (59). Additional research indicates that phytochemicals can selectively regulate multiple signaling pathways at the level of transcription, especially those involving mitogen-activated protein kinase (60). In this regard, in the study by Joseph et al (56), significant increases in several indices of neuronal signaling (eg, muscarinic receptor sensitivity), as well as reversals in age-related dysregulation in $^{45}\text{Ca}^{2+}$ -buffering capacity, an important index of neuronal dysfunction in aging (1), were observed in the diet groups but not in the control group.

Additional evidence for these signaling changes was seen in a recent study (61) in which APP/PS1 transgenic mice, which have genetic mutations that promote the production of β -amyloid and then hallmark Alzheimer disease-like plaques in several brain regions, were given blueberry supplements (as in reference 56). The supplementation was begun at 4 mo of age and continued until the mice reached 12 mo of age. The mice were then tested for their performance in a Y-maze. The results indicated that the blueberry supplement-treated mice showed performance similar to that of nontransgenic mice and significantly better than that of non-supplement-treated transgenic animals. Examination of the brains of the mice revealed no differences between the supplement-treated and non-supplement-treated APP/PS1 mice in the number of plaques. It appeared that there was a

discrepancy between plaque deposition and Y-maze performance, because performance did not decline among the blueberry supplement-treated APP/PS1 mice but did show decrements among the APP/PS1 mice maintained on the control diet.

Additional analyses revealed that the blueberry supplement-treated APP/PS1 mice showed higher concentrations of hippocampal ERK, as well as striatal and hippocampal protein kinase C α , than did the APP/PS1 mice maintained on a control diet. Both protein kinase C and ERK have been shown to be important in the conversion of short-term memory to long-term memory (62). These findings suggested that blueberry supplementation might prevent cognitive deficits by directly enhancing neuronal signaling and offsetting any putative deleterious effects of amyloid deposition. The data also revealed that blueberry supplementation, in addition to enhancing mitogen-activated protein kinase signaling, increased the sensitivity of muscarinic receptors (increasing striatal, carbachol-stimulated, GTPase activity). As mentioned above, these receptors have been implicated in cognitive function for many years (8).



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